SEDAR62-WP-17: Do Sagittal Otoliths Provide More Reliable Age Estimates Than Dorsal Spines for Gray Triggerfish?

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Do Sagittal Otoliths Provide More Reliable Age Estimates Than Dorsal Spines for Gray Triggerfish?

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Introduction

Gray triggerfish, *Balistes capriscus*, traditionally have been aged via counts of translucent zones in first dorsal spines due to the fact their otoliths (Fig. 1) are small, fragile, and difficult to extract and prepare for ageing (Johnson and Saloman 1984; Ingram 2001; Bernardes 2002; Allman et al. 2018). However, translucent zones in spines are difficult to interpret, occur in doublets, and often >10% of spine sections are deemed to be unreadable due to unclear translucent zones. Therefore, questions about potential ageing error have persisted with respect to ageing gray triggerfish with spine sections.

The concept of ageing error often is used to refer to both bias and imprecision, but those terms represent different sources of uncertainty. The accuracy of age estimates is typically assessed via age validation or verification studies (Camapana 2001). In the case of validation, growth zones in known-age fish are counted and the degree of bias is assessed. In the case of verification, marginal increment or condition (opaque versus translucent zones on otolith edge) analysis typically is performed to assess whether a single growth zone is formed annually in a given ageing structure. For gray triggerfish, Allman et al. (2016) reported marginal condition analysis revealed two peaks in translucent zone formation, which the authors inferred reflected the doublet pattern (two closely spaced translucent zones) interpreted as representing a single year in the life of a fish (Johnson and Saloman 1984; Ingram 2001). A tank-reared gray triggerfish injected with oxytetracycline also had one translucent zone form in vertebral, dorsal spine, and anal fin ray sections (Allman et al. 2016). While those findings are consistent with one growth zone formed each year, examination of a single fish does not represent comprehensive age validation for gray triggerfish.

Ageing precision, or repeatability, is often evaluated via the index of average percent error (APE), which is a measure of agreement in age estimates among two or more agers. A low APE typically imparts some confidence in age estimates, but in reality, a low APE means two or more readers independently counted roughly the same number of growth zones in a given ageing structure, such as a gray triggerfish dorsal spine section. However, if the growth zones in the

ageing structure do not accurately reflect the age of the fish, then it is irrelevant how reproducible biased counts are.

Production ageing laboratories in the southeastern US typically set an APE tolerance for reef fishes to be < 5%, which follows the guidance of Camapana (2001). The APE for some species, such as red snapper, is typically well below that threshold, while for others the APE among readers can be > 10%, and it is not unusual for the APE between readers of gray triggerfish dorsal spines to approach 15%. In the three most comprehensive gray triggerfish age and growth studies, Burton et al. (2015) and Kelly-Stormer et al. (2017) estimated an APE of 9-12% between readers of gray triggerfish spine sections from fish sampled in US Atlantic waters, while Allman et al. (2018) estimated an APE of 10.8% between readers for fish sampled in the US Gulf of Mexico (GOM). The ultimate cause of these high APEs, and the rationale for finding them acceptable, is often ascribed to higher than average variability in size at age, hence individual growth, reported for species such as vermilion snapper or gray triggerfish, with the thinking being that variable growth is reflected in differences in patterns or appearance that make otolith or dorsal spine microstructure difficult to interpret. However, difficulty in reading otolith or spine sections can manifest itself as ageing bias in addition to increasing APE estimates.

The purpose of this paper is to report results of two studies aimed at examining ageing error as well as the efficacy of utilizing sagittal otoliths to estimate age in gray triggerfish. In the first study, age estimates derived from otolith, spine, and vertebral sections of gray triggerfish sampled in US Atlantic waters were compared to examine agreement among ageing structures (Shervette and Dean 2015). In the second study, age estimates derived from spines and otoliths of fish sampled in the northeastern Gulf of Mexico (GOM) were validated via a novel application of the bomb radiocarbon chronometer. Results presented herein should be viewed as preliminary. However, they may have important implications for age-based stock assessment of gray triggerfish in both sampling regions.

Methods

US Atlantic Gray Triggerfish

Sampling

Gray Triggerfish were sampled during 2012-15 from South Carolina commercial catches. Fish were kept on ice until fishing vessels returned from offshore. Fish were filleted by the fishers and then the carcasses were placed back on ice until transfer to a -10 ºC freezer, where they remained until being processed for life history samples. Once thawed, date of capture was recorded for each sample and each carcass was measured to the nearest mm standard (SL), fork (FL), and total (TL) length. Sex was determined via macroscopic examination of gonads. The first dorsal spine was removed from each carcass, cleaned of excess tissue, and stored dry until further processing. Sagittal otoliths were dissected with vestibular organs intact from the head of each carcass via a notch cut into the cranium. Otoliths were then gently cleaned of adhering tissue and stored on plastic microcentrifuge tubes. Lastly, the penultimate caudal vertebra was removed from each carcass, cleaned, and stored dried in a paper envelop.

Age Estimation

Each spine sample was prepared for ageing by cutting two transverse sections immediately distal to the condyle groove (~0.6 mm thickness) using a low-speed saw with a diamond-edged double blade system (Ingram 2001). Sections were affixed to glass slides with Cytoseal™ epoxy, which was also utilized to cover sections. Prepared sections were viewed with a dissecting microscope at 10-20x magnification and transmitted light. Translucent zones were counted in spine sections to estimate age.

Otoliths were submerged in water with the cauda facing down and read whole. Fish age was estimated for each otolith by counting the number of opaque zones viewed at 20-50x magnification using a dissecting microscope with reflected light. Vertebrae were sectioned along their midsagittal axis to a thickness of 0.7 mm with a low-speed saw and then sections were mounted on glass slides with Cytoseal™ and translucent zones counted as described above for spines.

For each structure, two independent readers counted translucent or opaque zones for a sample without knowledge of fish length or date of capture. Spine and vertebral translucent zone counts, or whole otolith opaque zone counts, that differed between readers were reevaluated and a consensus count was recorded as the final age estimate.

US Gulf of Mexico Gray Triggerfish

Sampling and Age Estimation

Gray triggerfish were sampled during 2016-17 via fishery-independent sampling in the northeastern GOM. Fish were measured to FL, sex determined via macroscopic examination of gonads, and dorsal spines and otoliths extracted as described above. A subset of 20 fish was randomly selected from the full size range of samples. Age estimates derived from dorsal spine sections and whole otoliths were produced for these fish as described above.

Bomb Radiocarbon Age Validation

The bomb radiocarbon (^{14}C) chronometer was employed to validate age estimation based on dorsal spines translucent zone and otolith opaque zone counts in GOM gray triggerfish samples (n = 20). The principle behind this approach is the rapid increase in oceanic ^{14}C that resulted from atmospheric testing of nuclear weapons during the 1950s and 1960s created a biogeochemical tracer that serves as a chronometer (Broecker and Peng 1982). The bomb ^{14}C was incorporated into the aragonite (biogenic $CaCO_3$) skeletons of hermatypic corals (e.g., Druffel 1980,; Grottoli and Eakin 2007). Corals can be aged similar to fishes by counting growth zones in skeletal sections. Therefore, year-specific coral $\Delta^{14}C$ (a measure ^{14}C relative to a standard; see Approach and Methods) provides a chronometer to evaluate age estimates of other aragonite structures, such as fish otoliths (Kalish 1993).

After peaking in the mid 1970s, coral $\Delta^{14}C$ has declined around the globe as ^{14}C mixes out of the surface layer and into the deep ocean. Several authors have commented that the decline in coral $\Delta^{14}C$, which is a proxy for $\Delta^{14}C$ of dissolved inorganic carbon in the ocean, has declined linearly since approximately 1980. Andrews et al. (2013) compiled a regional coral $\Delta^{14}C$ time series for the northwestern Atlantic Ocean (latitude: 18-40 °N, longitude: 64-98 °W) which clearly demonstrates this post-1980 linear trend. Barnett et al. (2018) extended the linear portion of the time series to 2015 based on regional coral and known-age GOM red snapper samples (Fig. 2).

Application of the bomb ^{14}C chronometer to fish age validation typically involves coring adult otoliths to reveal aragonite formed during the first year of a fish's life, analyzing that aragonite for $\Delta^{14}C$ with accelerator mass spectrometry (AMS), and then overlaying the birth year estimate (sample year minus opaque zone count) and $\Delta^{14}C$ values from sampled fish on the reference (coral plus known-age red snapper samples in the GOM) time series. Visual inspection of the correspondence between the reference time series and samples from the species of interest is often conducted as a means to validate age estimation procedures, with close correspondence indicating accurate ageing. However, Kastelle et al. (2008) proposed fitting a function to the reference time series and then computing residuals between age validation samples and predicted $\Delta^{14}C$ for birth years in the dataset. Ageing bias is then assessed by advancing or declining all ages (birth years) by a set number of years and computing the sum of squared residuals (SSR) for each scenario, including the null model. The scenario with the lowest SSR is the most parsimonious solution.

Unfortunately, gray triggerfish otoliths are so small they are unable to be cored to produce a sufficient sample (1 mg of aragonite or 100 µg of C) for a robust AMSanalysis of birth year $\Delta^{14}C$. However, eye lenses, like otoliths, are not subjected to physiological reworking once formed. They are also composed of protein that is 50% C. Therefore, eye lens cores, which for gray triggerfish are ~5 mg corresponding to the mean mass of age-0 lenses, can be utilized as the birth year $\Delta^{14}C$ given $\Delta^{14}C$ undergoes no metabolic fractionation. Therefore, we sampled eyes from all the GOM fish, placed them in foil, and froze them at -80 ºC until core extraction. Cores were extracted by first dissecting lenses from eyes and then allowing them to dry in air; all tools that came in contact with lenses and the aluminum foil they were placed on had been combusted at 500 ºC to remove any C from their surfaces. As lenses dry they fracture and reveal inner laminae, or eye lens layers. Outer layers of laminae are then removed to reveal eye lens cores. Target core sizes and masses (~5 mg) were estimated based on age-0 (100-150 mm FL) triggerfish sampled during the SEAMAP Fall Groundfish Survey.

Eye lens cores were placed in combusted glass vials with foil liners and shipped to the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility at the Woods Hole Oceanographic Institution. Samples were analyzed for $\Delta^{14}C$ while correcting for $\delta^{13}C$. Data are reported in the standard permil notation. Data were plotted on the linear portion of the regional coral plus known-age red snapper $\Delta^{14}C$ time series utilizing both spine-derived and otolith-derived age, hence birth year, estimates. The method of Kastelle et al. (2008) was then applied to estimate whether age estimation was biased when utilizing either ageing structure.

Results and Discussion

There were 72 gray triggerfish samples from US Atlantic waters for which spine sections, vertebral sections, and whole otoliths all produced age estimates. Examination of vertebra- versus otolith-derived age estimates indicates a close agreement between those two structures (Fig. 3A). The same comparison between spine- and otolith-derived ages indicates spine sections routinely produced lower age estimates (Fig. 3B). This under-ageing of spine relative to otolith (and therefore vertebral) sections could be due to process error (i.e., translucent zones are not formed in spines each year) or measurement error (i.e., translucent zones were present but undetected). Given the general clarity of the sections and the experience of the personnel

preparing spine sections, we feel the latter is unlikely. However, Allman et al. (2016) reported strong agreement between translucent zone counts in spine versus vertebral sections from GOM fish. Overall, these results suggest uncertainty exists in spine-derived age estimates for gray triggerfish, although the cause (process versus measurement error) cannot be determined at this stage.

Results from application of the bomb ^{14}C chronometer to validate age estimation in gray triggerfish suggests sagittal otoliths are the more reliable aging structure. Plots of eye lens core $\Delta^{14}C$ versus spine-derived year of formation (birth year) estimates demonstrated much greater variance around the predicted line fit to the regional coral and red snapper time series than was observed for the otolith-derived estimates (Fig. 4). Furthermore, all the otolith-based data were within the 95% prediction intervals of the linear reference function (Fig. 4B), while several of the spine-based data points were outside the prediction intervals (Fig. 4A).

Application of the Kastelle et al. (2008) model to both data types presents an even stronger case that otoliths are more reliable structures to estimate age (Figs. 5&6). For the spine-derived age estimates, the +1 year scenario provided the lowest SSR, meaning under-ageing by one year on average with spine sections is likely. However, even with the addition of a year to the spine-derived age estimates (i.e., reducing birth year by 1) some of the data remain outside the 95% predictions intervals of the linear reference function (Fig. 5). Not only do the spine-derived age estimates appear biased, but they are also less precise than the otolith- derived age estimates, for which the null model was the most parsimonious fit and there was lower variance in the data.

Analyses and results described here should be viewed as preliminary. This work is being expanded to look at ageing error more comprehensively in gray triggerfish with NMFS-Cooperative Research Program funding. In that work, we have sampled 453 gray triggerfish to date, with a target of 1,000 fish that will be aged with otoliths, spines, and vertebrae. We are in the process of employing nano computerized tomography (CT) analysis of whole sagittae to 1) directly age fish based on 3-dimensional density gradients in otolith opaque versus translucent zones, and 2) model the 3-dimensional growth planes of the small but complex structures such that the most reliable age estimates can be produced. Radiocarbon age validation also will be enhanced with greater sample sizes (n = 60 additional samples). Therefore, a more complete picture of gray triggerfish ageing error, with respect to both bias and imprecision, should emerge from this work over the next several months.

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Figures

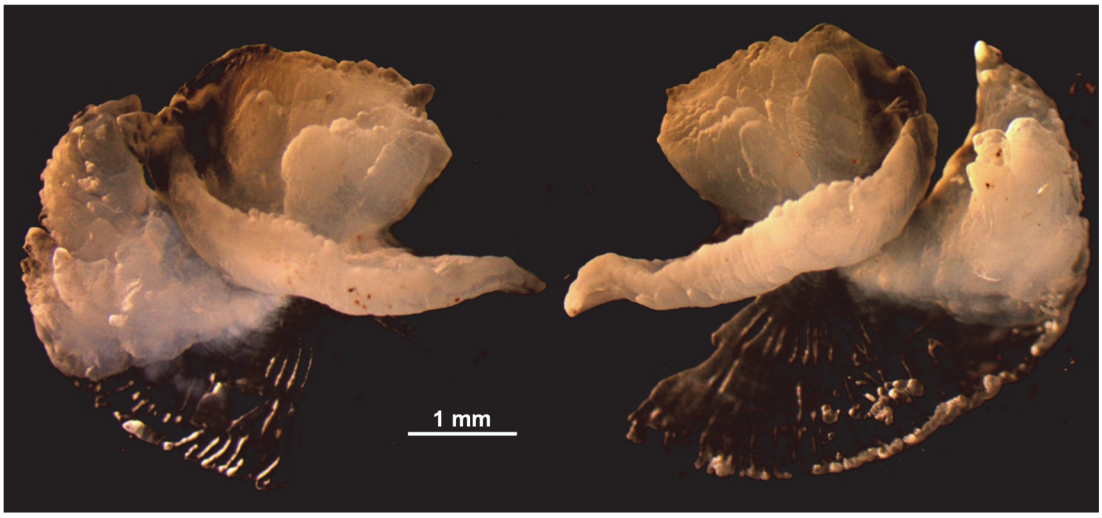

Figure 1. Digital images of a pair of gray triggerfish sagittal otoliths viewed with a light microscope and demonstrating their small size and fragile nature.

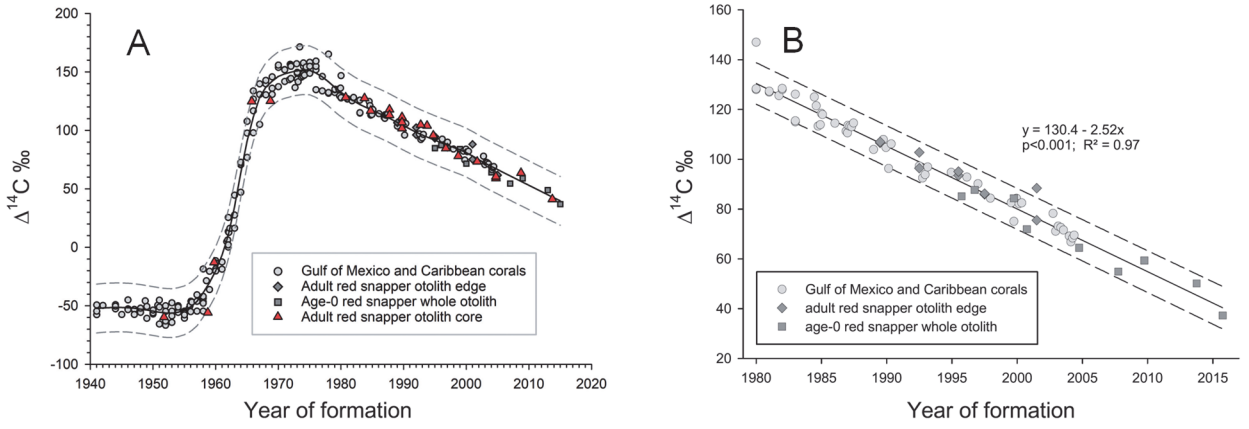

Figure 2. A) Scatterplot of regional coral plus known-age Gulf of Mexico red snapper (adult edge and whole age-0) otolith $\Delta^{14}C$ values versus year of formation. The solid line is a loess regression fit to the combined data; dashed lines are 95% prediction intervals. Adult red snapper otolith core data plotted for samples that were undergoing age validation. B) Linear regression fit to coral and known-age red snapper otolith $\Delta^{14}C$ values versus year of formation for the linear decline portion of the time series (source for both: Barnett et al. 2018).

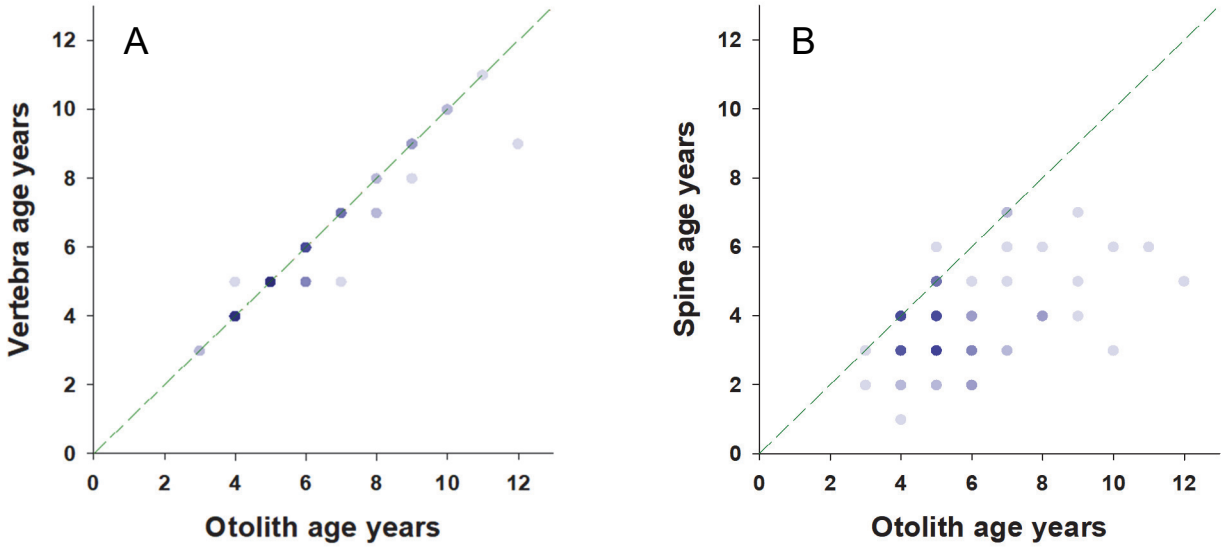

Figure 3. Age estimate comparisons between A) vertebral and otolith sections and B) spine and otolith sections for gray triggerfish sampled in US Atlantic waters. In each plot, n = 72. Symbol shading indicates sample size, which ranges from 1 (lightest) to 12 (darkest) age pairs.

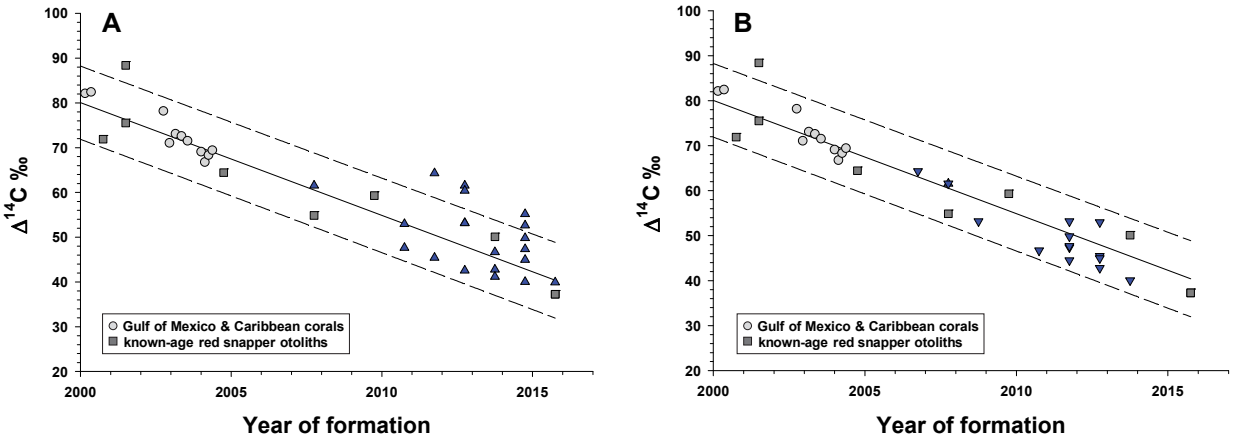

Figure 4. Gulf of Mexico gray triggerfish eye lens core $\Delta^{14}C$ (blue triangles) versus estimated year of formation (birth year) for A) spine-derived and B) otolith-derived age estimates overlain on regional coral and known-age red snapper otolith samples. Solid line is linear regression fit to coral and red snapper time series. Dashed lines are 95% prediction intervals.

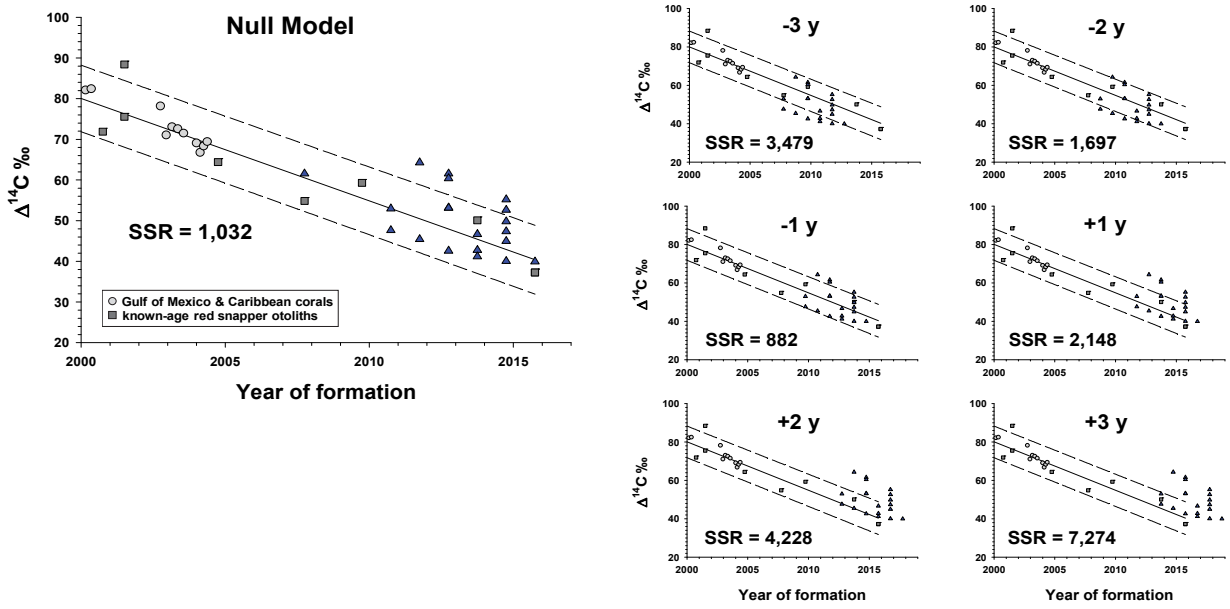

Figure 5. Results from Kastelle et al. (2008) approach to estimate ageing bias based on the bomb radiocarbon chronometer for Gulf of Mexico gray triggerfish spine-derived age estimates.

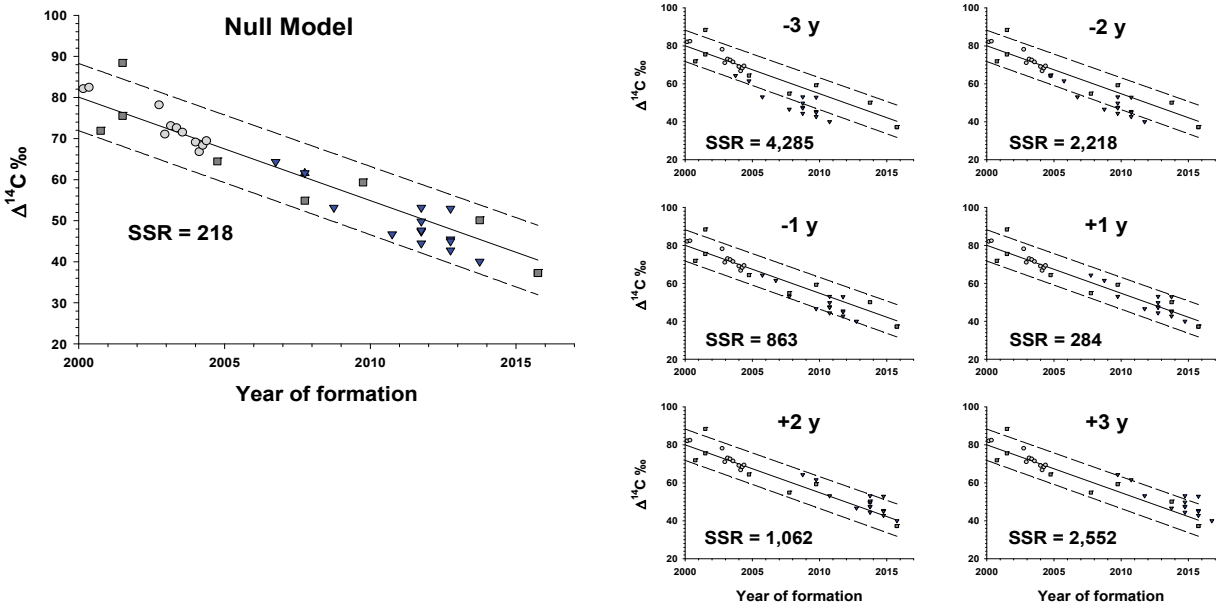

Figure 6. Results from Kastelle et al. (2008) approach to estimate ageing bias based on the bomb radiocarbon chronometer for Gulf of Mexico gray triggerfish spine-derived age estimates.